PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07K 14/435, 14/475, 14/705, A61K 38/18, 38/17, A61P 3/04, 5/04, C12N 15/12, C12Q 1/68

A1

(11) International Publication Number: WO 00/64931

(43) International Publication Date: 2 November 2000 (02.11.00)

(21) International Application Number: PCT/AU00/00342

(22) International Filing Date: 19 April 2000 (19.04.00)

(30) Priority Data:

PP 9919	23 April 1999 (23.04.99)	AU
PQ 6454	24 March 2000 (24.03.00)	AU

(71) Applicant *(for all designated States except US)*: AUTOGEN PTY LTD [AU/AU]; 210 Kings Way, South Melbourne, Victoria 3205 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: COLLIER, Greg [AU/AU]; 22 Kestrel Place, Ocean Grove, Victoria 3226 (AU). WALDER, Ken [AU/AU]; 54 Lake Avenue, Ocean Grove, Victoria 3226 (AU). ZIMMET, Paul [AU/AU]; 24 Linlithgow Road, Toorak, Victoria 3142 (AU).

(74) Agents: E JOHN L HUGHES et al.; Davies Collison Cave, Level 3, 303 Coronation Drive, Milton, Queensland 4064 (AU).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A LIGAND OF THE PROTEIN "BEACON"

(57) Abstract

The present invention relates generally to a ligand for a protein associated with modulating obesity, diabetes and metabolic energy levels in animals and humans and to genetic sequences encoding the ligand. More particularly, the present invention is directed to a ligand of the protein "beacon" and its homologues. The identification of the ligand molecule permits the development of a range of therapeutic and diagnostic protocols for obesity, diabetes and energy imbalance.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

A LIGAND OF THE PROTEIN "BEACON"

FIELD OF THE INVENTION

The present invention relates generally to a ligand for a protein associated with modulating obesity, diabetes and metabolic energy levels in animals and humans and to genetic sequences encoding the ligand. More particularly, the present invention is directed to a ligand of the protein "beacon" and its homologues. The identification of the ligand molecule permits the development of a range of therapeutic and diagnostic protocols for obesity, diabetes and energy imbalance.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical, veterinary and allied human and animal health fields. This is particularly the case in the investigation of the genetic bases involved in the etiology of certain disease conditions. One particularly significant condition from the stand point of morbidity and mortality is obesity and its association with non-insulin-dependent diabetes mellitus (NIDDM) and cardiovascular disease.

Obesity is defined as a pathological excess of body fat and is the result of an imbalance between energy intake and energy expenditure for a sustained period of time. Obesity is the most common metabolic disease found in affluent societies. The prevalence of obesity in these nations is alarmingly high, ranging from 10% to upwards of 50% in some subpopulations (Bouchard, 1994). Of particular concern is the fact that the prevalence of obesity appears to be rising consistently in affluent societies and is now increasing rapidly in less prosperous nations as they become more affluent and/or adopt cultural practices from the more affluent countries (Zimmet, 1992).

In Australia, for example, studies using the definition of obesity of BMI>30 have found prevalence rates for obesity of 8.2-9.3% in men and 9.1-11.1% in women (Risk Factor Prevalence Study Management Committee, 1990; Waters and Bennett, 1995). The prevalence rates for obesity are increasing in Australia, as they are in many affluent societies. Bennett and Magnus (1994) found that the mean weight of Australian females aged 20-69 increased by 3.1 kg (from 61.7 to 64.8 kg) from 1980 to 1989, while the corresponding increase in males was 1.8 kg (from 77.0 to 78.8 kg). No change in height was observed during this period. Accordingly, the crude prevalence rates of obesity increased from 8.0 to 13.2% in females and from 9.3 to 11.5% in males (Bennett and Magnus, 1994). All of the above changes were statistically significant ($p<0.05$).

The high and increasing prevalence of obesity has significant health implications. Obesity has been identified as a key risk indicator of preventable morbidity and mortality due to disease such as NIDDM and cardiovascular disease (National Health and Medical Research Council, 1996). The annual costs of obesity in Australia, for example, associated with these and other disease conditions have been conservatively estimated at AU$810 million (National Health and Medical Research Council, 1996).

A genetic basis for the etiology of obesity is indicated *inter alia* from studies in twins, adoption studies and population-based analyses which suggest that genetic effects account for 25-80% of the variation in body weight in the general population (Bouchard 1994; Kopelman *et al*, 1994; Ravussin, 1995). It is considered that genes determine the possible range of body weight in an individual and then the environment influences the point within this range where the individual is located at any given time (Bouchard, 1994).

Obesity is a complex and heterogeneous disorder and of considerable relevance to society. However, despite numerous studies into genes thought to be involved in the pathogenesis of obesity, there have been surprisingly few significant findings in this area. In addition, genome-wide scans in various population groups have not produced definitive evidence of the chromosomal regions having a major effect on obesity.

The hypothalamus has long been recognized as a key brain area in the regulation of energy intake. Early studies led to the dual-centre hypothesis which proposed that two opposing centres in the hypothalamus were responsible for the initiation and termination of eating, the lateral hypothalamus (LHA; "hunger centre") and ventromedial hypothalamus (VMH; "satiety centre"; Stellar, 1954). The dual-centre hypothesis has been repeatedly modified to accommodate the increasing information about the roles played by various other brain regions, neurotransmitter systems, and hormonal and neural signals originating in the gut on the regulation of food intake. In addition to the LHA and VMH, the paraventricular nucleus (PVN) is now considered to have an important integrative function in the control of energy intake.

A large number of neurotransmitters have been investigated as possible hypothalamic regulators of feeding behaviour including neuropeptide Y (NPY), glucagon-like peptide 1 (GLP-1), melanin-concentrating hormone (MCH), serotonin, cholecystokinin and galanin. Some of these neurotransmitters stimulate food intake, some act in an anorexigenic manner and some have diverse effects on energy intake depending on the site of administration. For example, gamma-aminobutyric acid (GABA) inhibits food intake when injected into the LHA, but stimulates eating when injected into the VMH or PVN (Leibowitz, 1985). Feeding behaviour is thought to be greatly influenced by the interaction of stimulatory and inhibitory signals in the hypothalamus.

In work leading up to the present invention, the inventors made a significant break through in determining a genetic basis of obesity by identifying a genetic sequence referred to as *"beacon"* which is differentially expressed in lean and obese animals. This genetic sequence is associated with energy balance and is also involved in modulating obesity and diabetes. See International Patent Application No. PCT/AU98/00902 filed on 30 October, 1998 in the names of International Diabetes Institute and Deakin University. This International application is incorporated herein by reference. The inventors now propose the presence of a ligand capable of interacting with the protein, beacon. The interaction between beacon and its ligand is proposed to be a factor in obesity, diabetes and energy imbalance. The identification of a beacon-interacting ligand provides the means for developing a range of therapeutic and diagnostic agents for conditions such as obesity and diabetes.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier, i.e. <400>1, <400>2, etc. A sequence listing is provided at the end of the description.

Accordingly, one aspect of the present invention provides a ligand of a protein or a derivative, homologue, analogue or mimetic of said protein which protein is produced in larger amounts in hypothalamus tissue of obese animals compared to lean animals.

Another aspect of the present invention provides a ligand or a derivative, homologue, analogue or mimetic which ligand is capable of interacting with a protein which is produced in a larger amount of hypothalamus tissue of obese animals compared to lean animals and which is encoded by a nucleotide sequence substantially as set forth in <400>1 or <400>4 or a nucleotide sequence having at least about 50% similarity thereto or a nucleotide sequence capable of hybridizing <400>1 or <400>4 under low stringency conditions.

Yet another aspect of the present invention is directed to a ligand capable of interacting with a protein which comprises the amino acid sequence substantially as set forth in <400>2 or <400>5 or an amino acid sequence having at least 50% similarity thereto and wherein said protein is produced in larger amounts in hyperthalamus tissue of obese animals compared to lean animals.

In still another aspect of the present invention, the nucleotide sequence substantially as set forth in <400>6 and/or <400>7 or a nucleotide sequence having at least about 50% similarity to one or both of <400>6 or <400>7 after optimal alignment or a nucleotide sequence capable of hybridizing to one or both of <400>6 or <400>7 under low stringency conditions.

In still yet another aspect of the present invention, the ligand comprises a nucleotide sequence substantially as set forth in Figure 2 or Figure 3 or a nucleotide sequence having at least about 50% similarity to a nucleotide sequence in Figure 2 or Figure 3 after optimal alignment or a nucleotide sequence capable of hybridizing to a nucleotide sequence in Figure 2 or Figure 3 under low stringency conditions.

Another aspect of the present invention contemplates a method of identifying a ligand of the protein beacon or its derivatives, said method comprising introducing a first genetic construct in a yeast strain, said genetic construct comprising a nucleotide sequence encoding all or part of beacon fused to a nucleotide sequence encoding one of a DNA binding (DB) domain or an activation domain (AD) and introducing a second genetic construct into said yeast comprising a cDNA, said second genetic construct comprising elements of a cDNA library fused to a nucleotide sequence encoding the other of a DB domain or AD domain and selecting yeast cells which comprise both genetic constructs and in which a reporter gene has been subjected to two-hybrid dependent transcription.

Yet another aspect of the present invention contemplates a method for modulating expression of *beacon* ligand in a mammal, said method comprising contacting the *beacon* ligand gene with an effective amount of a modulator of *beacon* ligand expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *beacon* ligand.

Still another aspect of the present invention contemplates a method of modulating activity of beacon in a mammal, said method comprising administering to said mammal a modulating effective amount of a soluble *beacon* ligand or a derivative thereof for a time and under conditions sufficient to increase or decrease beacon activity.

Still yet another aspect of the present invention contemplates, in one embodiment, a composition comprising a soluble form of *beacon* ligand or a modulator of *beacon* ligand expression and one or more pharmaceutically acceptable carriers and/or diluents.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a representation showing the nucleotide sequence of both strands of a differentially expressed band in hypothalamus tissue of lean and obese *Psammomys obesus* corresponding to *beacon*. The amino acids encoded by each codon are shown above in single letter code and the numbering refers to the amino acid position from the start codon.

Figure 1B is a representation of a nucleotide and corresponding amino acid sequence of the short form of beacon. Note that amino acid 15 may be His or Arg and the corresponding codon may be CGC or CAC, respectively.

Figure 2 is a representation of the partial nucleotide sequence of pPC86 clone 31 which encodes a beacon ligand.

Figure 3 is a representation of multiple sequence alignments of pPC86 clone 31, which encodes a beacon ligand, with CLK4M, STYMA and CLK1H.

Figure 4 is a diagrammatic representation of the yeast two-hybrid screening protocol.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification of a ligand for the product of a gene associated *inter alia* with regulation of energy balance, obesity and diabetes. The preferred gene is referred to as "beacon" and was identified following differential screening of hypothalamic mRNA between lean and obese animals (see International Patent Application No. PCT/AU98/00902).

The term "ligand" means a peptide, polypeptide or protein which binds, forms a close interaction to or which otherwise associates with a protein involved in energy imbalance, obesity and diabetes. Examples of ligands contemplated by the present invention include cell bound receptors, soluble receptors, intracellular ligands, extracellular ligands and partners in a complex comprising the protein involved in energy imbalance, obesity and diabetes. A single ligand may be involved in interaction with the protein or a complex of two or more ligands may be required to from a complex with the subject protein. The term "ligand" also includes binding or interacting partners, cell bound receptors and soluble receptors.

The terms "lean" and "obese" are used in their most general sense but should be considered relative to the standard criteria for determining obesity. Generally, for human subjects the definition of obesity is BMI>30 (Risk Factor Prevalence, 1990; Waters and Bennett, 1995).

Conveniently, an animal model may be employed to study the effects of obese and lean animals. In particular, the present invention is exemplified using the *Psammomys obesus* (the Israeli sand rat) animal model of dietary-induced obesity and NIDDM. In its natural desert habitat, an active lifestyle and saltbush diet ensure that they remain lean and normoglycemic (Shafrir and Gutman, 1993). However, in a laboratory setting on a diet of *ad libitum* chow (on which many other animal species remain healthy), a range of pathophysiological responses are seen (Barnett *et al.*, 1994a, b; Barnett *et al.*, 1995). By the age of 16 weeks, more than half of the animals become obese and approximately one-third develop NIDDM. Only hyperphagic animals go on to develop hyperglycemia, highlighting the importance of excessive energy intake in the pathophysiology of obesity and NIDDM in *Psammomys obesus* (Collier *et al.*, 1997a; Walder

et al., 1997a). Other phenotypes found include hyperinsulinemia, dyslipidemia and impaired glucose tolerance (Collier *et al.*, 1997a, b). *Psammomys obesus* exhibit a range of bodyweight and blood glucose and insulin levels which forms a continuous curve that closely resembles the patterns found in human populations, including the inverted U-shaped relationship between blood glucose and insulin levels known as "Starling's curve of the pancreas" (Barnett *et al.*, 1994a; DeFronzo, 1988). It is the heterogeneity of the phenotypic response of *Psammomys obesus* which make it an ideal model to study the etiology and pathophysiology of obesity and NIDDM.

A preferred aspect of the present invention is directed to a ligand capable of interacting with "beacon", the product of the gene *"beacon"*. The nucleotide sequence of *beacon* is set forth in <400>1 and <400>4. The amino acid sequence of beacon is set forth in <400>2 and <400>5.

Accordingly, another aspect of the present invention provides a ligand or a derivative, homologue, analogue or mimetic which ligand is capable of interacting with a protein which is produced in a larger amount of hypothalamus tissue of obese animals compared to lean animals and which is encoded by a nucleotide sequence substantially as set forth in <400>1 or <400>4 or a nucleotide sequence having at least about 50% similarity thereto or a nucleotide sequence capable of hybridizing <400>1 or <400>4 under low stringency conditions.

According, another aspect of the present invention is directed to a ligand capable of interacting with a protein which comprises the amino acid sequence substantially as set forth in <400>2 or <400>5 or an amino acid sequence having at least 50% similarity thereto and wherein said protein is produced in larger amounts in hyperthalamus tissue of obese animals compared to lean animals.

Reference herein to similarity is generally at a level of comparison of at least 15 consecutive or substantially consecutive nucleotides or at least 5 consecutive or substantially consecutive amino acid residues. Generally, similarity or identity is determined after optimal alignment of the sequences.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity.

Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence similarity", "sequence identity", "percentage of sequence similarity", "percentage of sequence identity", "substantially similar" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 or above, such as 30 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e. only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.* (1997). A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.* (1998).

The terms "sequence similarity" and "sequence identity" as used herein refers to the extent that sequences are identical or functionally or structurally similar on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity", for example, is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g. A, T, C, G, I) or the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e. the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software. Similar comments apply in relation to sequence similarity.

Reference herein to a low stringency includes and encompasses from at least about 0 to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization, and at least about 1 M to at least about 2 M salt for washing conditions. Generally, low stringency is at from about 25-30°C to about 42°C. The temperature may be altered and higher temperatures used to replace formamide and/or to give alternative stringency conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization, and at least about 0.5 M to at least about 0.9 M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41$ (G+C)% (Marmur and Doty, 1962). However, the T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatch base pairs (Bonner and Laskey, 1974). Formamide is optional in these hybridization conditions. Accordingly,

particularly preferred levels of stringency are defined as follows: low stringency is 6 x SSC buffer, 0.1% w/v SDS at 25-42°C; a moderate stringency is 2 x SSC buffer, 0.1% w/v SDS at a temperature in the range 20°C to 65°C; high stringency is 0.1 x SSC buffer, 0.1% w/v SDS at a temperature of at least 65°C.

The nucleotide sequence or amino acid sequence of the beacon ligand of the present invention may correspond to exactly the same sequence of the naturally occurring ligand or its gene (or corresponding cDNA) or may carry one or more nucleotide or amino acid substitutions, additions and/or deletions.

Any number of approaches may be employed to identify the ligand.

In one particularly useful method, a yeast two-hybrid system is employed. The yeast two-hybrid system is an *in vivo* genetic technique that can be utilized for the identification of protein:protein interactions. The essence of the two-hybrid system is that interaction between two proteins (X and Y) can be identified by reconstituting active transcription factor dimers. In yeast, these dimers are formed between two fusion proteins, one of which contains a DNA binding (DB) domain fused to the first protein of interest X and the other, an activation domain (AD) fused to a second protein Y. Interaction between DB-X and AD-Y forms a functional transcription factor that activates chromosomally integrated-reporter genes driven by promoters containing the relevant DB binding sites. When a selectable marker such as *HIS3* is used as a reporter gene, two-hybrid dependent transcription activation can be monitored by growth on plates lacking histidine. This technique can, therefore, be applied to test whether two known proteins interact or to detect an unknown protein, encoded by a cDNA library, that interacts with a protein of interest.

Accordingly, another aspect of the present invention contemplates a method of identifying a ligand of the protein beacon or its derivatives, said method comprising introducing a first genetic construct in a yeast strain, said genetic construct comprising a nucleotide sequence encoding all or part of beacon fused to a nucleotide sequence encoding one of a DNA binding (DB) domain or an activation domain (AD) and introducing a second genetic construct into said

yeast comprising a cDNA, said second genetic construct comprising elements of a cDNA library fused to a nucleotide sequence encoding the other of a DB domain or AD domain and selecting yeast cells which comprise both genetic constructs and in which a reporter gene has been subjected to two-hybrid dependent transcription.

According to this embodiment, if the cDNA from the cDNA library encodes a binding partner for beacon, then a dimer forms and the DB and AD domains permit transcription of the reporter gene.

In one embodiment, the yeast reporter gene is *HIS3* although any other reporter gene may be employed. Preferably, the reporter gene provides a selectable marker.

In a particularly preferred embodiment, the ligand comprises the nucleotide sequence substantially as set forth in <400>6 and/or <400>7 or a nucleotide sequence having at least about 50% similarity to one or both of <400>6 or <400>7 after optimal alignment or a nucleotide sequence capable of hybridizing to one or both of <400>6 or <400>7 under low stringency conditions.

In another particularly preferred embodiment, the ligand comprises a nucleotide sequence substantially as set forth in Figure 2 or Figure 3 or a nucleotide sequence having at least about 50% similarity to a nucleotide sequence in Figure 2 or Figure 3 after optimal alignment or a nucleotide sequence capable of hybridizing to a nucleotide sequence in Figure 2 or Figure 3 under low stringency conditions.

For convenience, the ligand capable of interaction with beacon is referred to as "beacon ligand". The corresponding genetic sequence encoding beacon ligand is referred to herein as *"beacon* ligand". Reference herein to *beacon* ligand includes, where appropriate, reference to the genomic gene or cDNA as well as any naturally occurring or induced derivatives. Apart from the substitutions, deletions and/or additions to the nucleotide sequence, the present invention further encompasses mutants, fragments, parts and portions of the nucleotide sequence corresponding to the *beacon* ligand. The *beacon* gene itself may encode a "short" form or "long"

form of beacon. Both the long and short forms of beacon are biologically active, and suppression of the activity of either or both forms is included in this invention. The short form of beacon can be readily synthesized *in vitro*, while the long form can be produced using expression vectors. A beacon ligand may be any protein including a heat shock protein and/or a cdc-like protein. In a preferred embodiment, the beacon ligand is a cdc-like kinase with strong homology to mouse clk4.

A homologue of beacon ligand or *beacon* ligand is considered to be a ligand from another animal species. The *beacon* ligand gene is exemplified herein from *Psammomys obesus* hypothalamus. The invention extends, however, to the homologous gene, as determined by nucleotide sequence and/or function, from humans, primates, livestock animals (e.g. cows, sheep, pigs, horses, donkeys), laboratory test animals (e.g. mice, guinea pigs, hamsters, rabbits), companion animals (e.g. cats, dogs) and captured wild animals (e.g. rodents, foxes, deer, kangaroos).

Apart from the yeast two-hybrid method, the ligand of the present invention and, in particular, beacon ligand, may also be identifiable by a number of other means. In one method, beacon or a ligand binding portion thereof is labelled with a reporter molecule and used to screen cells, cell lysate and biological fluid (including blood, serum, lymph fluid) for binding to ligand. For cloning of *beacon* ligand, a cDNA library is conveniently prepared and expressed in a suitable cell such as CHO cells and the presence of beacon ligand is then determined by, for example, beacon or a ligand binding portion thereof labelled with a reporter molecule.

The identification of cell types having a beacon ligand is readily determined by incubated cells with beacon, with or without neuropeptide Y (NPY) or leptin and screening for an effect. Generally, the effect is the expression of select genes or screening for signal transduction or screening for phenotypic changes. Another useful technique involves the yeast two-hybrid system. This is particularly useful where the ligand is intracellular and not expressed on the cell surface.

The present invention provides the *beacon* ligand gene as well as a peptide, polypeptide or

protein encoded thereby. The nucleic acid molecule of this aspect of the present invention and in particular *beacon* ligand gene and its derivatives and homologues may be in isolated or purified form and/or may be ligated to a vector such as an expression vector. Expression may be in a eukaryotic cell line (e.g. mammalian, insect or yeast cells) or in microbial cells (e.g. *E. coli)* or both.

The derivatives of the *beacon* ligand nucleic acid molecule of the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable for use in co-suppression and fusion nucleic acid molecules. Ribozymes and DNA enzymes are also contemplated by the present invention directed to *beacon* ligand or its mRNA.

Reference herein to a beacon ligand includes reference to isolated or purified naturally occurring beacon ligand molecules as well as any derivatives, homologues, analogues and mimetics thereof. Derivatives includes parts, fragments and portions of a beacon partner as well as single and multiple amino acid substitutions, deletions and/or additions to the beacon partner.

Other derivatives of a beacon ligand include chemical analogues. Analogues of a beacon ligand contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose confirmational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic

condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 1.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib

D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu

N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

Crosslinkers can be used, for example, to stabilize 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

All such modifications may also be useful in stabilizing the beacon partner molecule for use in *in vivo* administration protocols or for diagnostic purposes.

The identification of a beacon ligand permits the generation of a range of therapeutic molecules capable of modulating expression of *beacon* or *beacon* ligand or modulating the activity of beacon or beacon ligand. Modulators contemplated by the present invention includes agonists and antagonists of *beacon* ligand expression. Antagonists of *beacon* ligand expression include antisense molecules, ribozymes and co-suppression molecules. Agonists include molecules which increase promoter activity or which interfere with negative regulatory mechanisms. Antagonists of beacon ligand include antibodies and inhibitor peptide fragments. All such molecules may first need to be modified to enable such molecules to penetrate cell membranes. Alternatively, viral agents may be employed to introduce genetic elements to modulate expression of a *beacon* ligand. Insofar as beacon acts in association with other genes such as the *ob* gene which encodes leptin, the therapeutic molecules of the present invention may target both the *beacon* ligand and *ob* ligand genes or their translation products.

The present invention contemplates, therefore, a method for modulating expression of *beacon* ligand in a mammal, said method comprising contacting the *beacon* ligand gene with an effective amount of a modulator of *beacon* ligand expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *beacon* ligand.

For example, a nucleic acid molecule encoding *beacon* ligand or a derivative or homologue thereof may be introduced into a cell to enhance the ability of that cell to produce *beacon* ligand. Conversely, *beacon* ligand antisense sequences such as oligonucleotides may be introduced to decrease the availability of *beacon* ligand molecules.

Another aspect of the present invention contemplates a method of modulating activity of beacon in a mammal, said method comprising administering to said mammal a modulating effective amount of a soluble *beacon* ligand or a derivative thereof for a time and under conditions sufficient to increase or decrease beacon activity. The derivative of *beacon* ligand may be a proteinaceous molecule or a chemical entity such as a product identified from a natural product library or chemical library.

One convenient means of screening for antagonists of beacon ligand when in the form of a receptor is to incubate a cell carrying a beacon ligand in the form of a receptor with beacon with or without a potential antagonist and screening for a differential effect when the antagonist is applied. Again, the effect may be gene expression, signal transduction and/or phenotypic changes.

Modulating levels of *beacon* ligand expression or beacon ligand activity is important in the treatment of a range of conditions such as obesity, anorexia, energy imbalance, diabetes, metabolic syndrome, dyslipidemia, hypertension and insulin resistance. It may also be useful in the agricultural industry to assist in the generation of leaner animals, or where required, more obese animals. Accordingly, the mammal contemplated by the present invention includes but is not limited to humans, primates, livestock animals (e.g. pigs, sheep, cows, horses, donkeys), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits), companion animals (e.g. dogs, cats) and captured wild animals (e.g. foxes, kangaroos, deer). A particularly preferred host is a human, primate or livestock animal.

Accordingly, the present invention contemplates in one embodiment a composition comprising a soluble form of beacon ligand or a modulator of *beacon* ligand expression and one or more pharmaceutically acceptable carriers and/or diluents. The compositions may also comprise leptin

or modulators of leptin activity or *ob* expression.

For brevity, all such components of such a composition are referred to as "active components".

The compositions of active components in a form suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

The carrier can be a solvent or other medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active components in the required amount in the appropriate solvent with optionally other ingredients, as required, followed by sterilization by, for example, filter sterilization, irradiation or other convenient means. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active molecules are suitably protected they may be orally administered, for example,

with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active

ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active component may be compounded for convenient and effective administration in sufficient amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. A unit dosage form can, for example, contain the principal active component in amounts ranging from 0.5 µg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 µg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

In general terms, effective amounts of active ingredient will range from 0.01 ng/kg/body weight to above 10,000 mg/kg/body weight. Alternative amounts range from 0.1 ng/kg/body weight is above 1000 mg/kg/body weight. Active ingredients may be administered per minute, hour, day, week, month or year depending on the condition being treated. The route of administration may vary and includes intravenous, intraperitoneal, sub-cutaneous, intramuscular, intranasal, *via* suppository, *via* infusion, *via* drip, orally or *via* other convenient means. Compositions may be formulated in a variety of ways and reference may be conveniently made to Remington Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Pennsylvania, USA.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable

of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating *beacon* ligand expression or beacon ligand activity. The vector may, for example, be a viral vector.

Still another aspect of the present invention is directed to antibodies to beacon ligand and its derivatives and homologues. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to beacon or may be specifically raised to beacon or derivatives or homologues thereof. In the case of the latter, beacon ligand or its derivatives or homologues may first need to be associated with a carrier molecule. The antibodies and/or recombinant beacon ligand or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents.

For example, beacon ligand and its derivatives can be used to screen for naturally occurring beacon or antibodies to beacon ligand which may occur in certain autoimmune diseases or where cell death is occurring. These may occur, for example in some autoimmune diseases. Alternatively, specific antibodies can be used to screen for beacon partner. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA.

Antibodies to beacon partner of the present invention may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to the beacon or may be specifically raised to the beacon or its derivatives. In the case of the latter, the beacon protein may need first to be associated with a carrier molecule. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool or as a means for purifying beacon ligand.

For example, specific antibodies can be used to screen for beacon partner proteins. The latter would be important, for example, as a means for screening for levels of beacon partner in a cell extract or other biological fluid or purifying beacon made by recombinant means from culture

supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of beacon partner.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of beacon partner or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art (see, for example, Douillard and Hoffman, 1981; Kohler and Milstein, 1975, Kohler and Milstein, 1976).

Another aspect of the present invention contemplates a method for detecting beacon or a derivative or homologue thereof in a biological sample from a subject said method comprising contacting said biological sample with a beacon ligand or a derivative or homologue thereof for a time and under conditions sufficient for a complex to form, and then detecting said complex.

The presence of the complex is indicative of the presence of beacon. This assay may be

quantitated or semi-quantitated to determine a propensity to develop obesity or other conditions or to monitor a therapeutic regimen.

Conveniently, beacon partner is immobilized to a solid support and a biological sample brought into contact with the immobilized molecule.

The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes to immobilize *beacon* partner are well-known in the art and generally consist of cross-linking, covalently binding or physically adsorbing the molecule to the solid support. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to about 37°C) to allow binding of beacon to is receptor. Following the incubation period, solid phase is washed and an antibody added directed to beacon. The presence of antibody binding is indicative of beacon being immobilized to its receptor.

Generally, either the beacon partner or an antibody to beacon is labelled with a receptor molecule.

In an alternative embodiment, beacon itself or a ligand binding portion thereof is labelled with a reported molecule and used to screen for beacon ligand.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

The present invention also contemplates genetic assays such as involving PCR analysis to detect

beacon ligand or its derivatives.

The present invention further extends to a clk ligand which is independent of beacon or beacon-clk interaction. The clk ligand is useful for a range of applications such as acting as an antagonist for clk interaction with other ligands. The clk ligand of this aspect of the present invention, for example, is the treatment of diabetes and/or other conditions associated with clk. This aspect of the present invention further contemplates nucleic acid molecules encoding the clk ligand as well as compositions comprising the clk ligand such as pharmaceutical compositions.

The present invention is further described by reference to the following non-limiting Figures and Examples.

A summary of sequence identifiers used throughout the subject specification is provided in Table 2.

TABLE 2

SEQUENCE IDENTIFIER	DESCRIPTION
<400>1	Nucleotide sequence for *beacon*
<400>2	Amino acid sequence for beacon
<400>3	Complementary sequence for <400>1
<400>4	Nucleotide sequence for human *beacon*
<400>5	Amino acid sequence for short form of beacon
<400>6	Partial nucleotide sequence of pPC86 clone 31 beacon ligand
<400>7	Partial nucleotide sequence of pPC86 clone 31 beacon ligand

A summary of the single and three letter abbreviations for amino acid residues used in the present specification is provided in Table 3.

TABLE 3

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

EXAMPLE 1

Animals

A *Psammomys obesus* colony is maintained at Deakin University, Geelong, Victoria, Australia, with the breeding pairs fed *ad libitum* a diet of lucerne and chow. Experimental animals were weaned at four weeks of age and given a diet of standard laboratory chow from which 12% of energy was derived from fat, 63% from carbohydrate and 25% from protein (Barastoc, Pakenham, Australia). Animals were housed individually in a temperature controlled room (22 ± 1°C) with a 12-12-hour light-dark cycle. The animals used in the study were aged 16-20 weeks.

EXAMPLE 2

Beacon ligand in cell lines

This example provides means for identifying a beacon ligand (e.g. beacon receptor) in cells lines which also helps in the study of signal transduction mechanisms. Examples of suitable cell lines include 3T2, GT1-7, HepG2 and primary cultures from Israeli Sand Rats. Cells are treated with beacon with and without neuropeptide Y(NPY) or leptin and an effect observed. Generally, approximately 3-5 genes are selected for changes in expression. Analysis is generally conducted in macro-or micro-assays. Once a cell is identified carrying a beacon receptor, a cDNA library is prepared and *beacon* ligand identified. Signal transduction studies may also be conducted (e.g. Ca^{2+}, cAMP, kinases, phosphatases).

EXAMPLE 3

Intracellular beacon ligand

In cases where a cell does not secrete beacon or synthesize it as a cellular receptor, the yeast two-hybrid system is useful for identifying beacon ligand as a binding partner of beacon.

EXAMPLE 4

Fusion protein fishing

Yeast Two-hybrid System

(using the ProQuest Two-hybrid System available from Life Technologies)

The steps employed are as follows:

- Clone the beacon gene in frame with the *GAL4* activation domain of the yeast vector pPC86. Libraries available include: expression human (brain and foetal brain), mouse (embryo 8.5 or 10 days, liver, brain and lymph node), C. elegans, HeLa cell and rat (liver and brain). A custom library *(Pssamomys obesus)* is also created.

- Transform the yeast strain MaV203 with the pDBLeu-beacon plasmid construct.

- Test for self activation of the pDBLeu-beacon fusion protein and determine the concentration of 3-Amino-1,2,4-Triazole (3AT) required to titrate basal HIS3 expression levels. HIS3 encodes imidazole glycerol phosphate deydratase, an enzyme involved in histidine biosynthesis. This enzyme is specifically inhibited in a dose-dependent manner by 3AT. To maximize sensitivity of the HIS3 reporter gene, strain MaV203 expresses a basal level of HIS3. By determining the threshold of resistance to 3AT and including that concentration of 3AT in plates lacking histidine, even slight increases in HIS3 reporter is detected, enhancing the likelihood of detecting even weak protein:protein interactions.

- Transform MaV203 cells containing the pDBLeu-beacon construct with pC86 library using antibiotic resistance (ampicillin and kanamycin) to select for cells that contain both plasmids and induce the HIS3 reporter gene.

- Purify cells containing candidate interacting proteins then patch isolated colonies onto a masterplate.

- Replica plate from the master plate onto selective plates to determine whether the three reporter genes are induced.

- For cells inducing the reporter genes confirm that DB-beacon and AD-fusion protein (from the cDNA library) interact when AD-fusion protein is retested with fresh DB-beacon plasmid by either a retransformation assay or a version of plasmid shuffling.

- Protein:protein interactions detected are confirmed by biological assays such as DNA sequencing of the cDNA clone to determine if the interacting protein has been previously identified. This fusion protein is also expressed in *E. coli* and co-precipitation experiments using antibodies raised against beacon or monoclonal antibodies raised against GAL4-AD domains (protein tags derived from the expression vectors). Other methods are employed to confirm that these proteins interact such as by surface receptor panning and fusion protein fishing.

A full length beacon sequence is cloned into the bacterial expression plasmid pGEX (Pharmacia Biotech). pGEX vectors allow for inducible, high level intracellular expression of genes as fusions with *Schistoma japonicum* glutathione S-transferase (GST). Induced bacterial cultures expressing pGEX-beacon are lysed by sonication in 50mM Tris-HCl (pH 7.4) containing 1% w/v Triton X-100, 1% v/v Tween 20, 2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, and 10 µg/ml aprotinin. Affinity resins for the isolation of beacon binding proteins are prepared by immobilizing GST-beacon onto glutathione-Sepharose 4B beads (Pharmacia). Similar resins are prepared using GST alone to act as a control for the specificity of interaction. Affinity resins are incubated in the presence of brain lysates (or other tissue lysates of interest) and after extensive washing, proteins bound to resins are released either by bioling in SDS sample buffer or by elution with Tris-HCl (pH 7.4) containing 0.5% v/v Triton X-100. Binding proteins are then separated by SDS-PAGE and visualized by silver staining or Coomassie Blue staining. Regions of the gel containing beacon binding proteins are excized and the gel slices digested and purified by anion-exchange and reverse phase HPLC prior to amino acid sequencing.

EXAMPLE 5

Yeast two-hybrid screening

The full length Beacon gene was cloned into the expression vector pDBLeu, which encodes the *GAL 4* DNA binding domain. To screen and identify potential proteins that interact with the 73aa Beacon gene product, a commercial human brain cDNA library was purchased. This cDNA expression library was constructed in the two-hybrid activation domain vector pPC86. Following vector construction, pDBLeu - Beacon (pDB73Be) was introduced into the yeast strain MaV203 by transformation. MaV203 cells containing pDB73Be were then used to introduce, by transformation, the pPC86 cDNA library. Candidate positive clones were identified by growth on media that selects for cells containing both plasmids as well as induction of the *HIS3* reporter gene. Greater than 10^6 transformants were screened for interaction with 73aa Beacon. Of this figure, 28 clones were identified as preliminary positives as a result of induction of the *HIS3* reporter gene.

Clones containing candidate interacting proteins were purified and re-tested for induction of three independent reporter genes. Of the 28 clones examined, three clones (clones 12, 16 and 31) were identified as containing potential interacting proteins due induction of all three reporter genes. To further ascertain the authenticity of interaction with 73aa Beacon, plasmid DNA from each clone was selectively isolated and re-introduced into MaV203. The re-transformation assay confirmed these clones as containing potential positive interactors with 73aa Beacon. Specifically, clones 12 and 16 were both identified as containing potential weak interacting proteins with 73aa Beacon. In contrast, clone 31 was shown to contain a strong interacting protein. The results are shown in Table 4. Plasmid DNA from each clone has been selectively isolated and partial sequences for the unknown cDNA's determined.

TABLE 4

Reporter gene expression

Clone	*-HIS*	*-URA*	X-gal	Result
Clone 12	+	+	White	+ve weak interacting proteins
Clone 16	+	+	White	+ve weak interacting proteins
Clone 31	+++	+++	Blue	+ve strong interacting proteins

Of the three clones that exhibited positive interaction with 73aa Beacon, clones 12 and 16 revealed overlapping partial cDNA sequences that were found to be 100% homologous to that of the human heat shock protein 2 (HSPB2) in the regions examined. HSPB2 belongs to the small heat shock protein (HSP20) family and has been shown to bind and activate the myotonic dystrophy protein kinase. *In vivo*, heat shock proteins have been shown to interact with large numbers of different proteins, consequently interaction of HSBP2 with 73aa Beacon may prove to be biologically irrelevant in the context of Beacon action.

Partial cDNA sequence data for clone 31 (<400>7) was found to be highly homologous to that for the gene encoding mouse cdc2/CDC28-like protein kinase 4 in the region examined (Table 5). This kinase is considered to be closely related to the yeast cdc2/CDC28 kinases that have been shown to regulate the cell cycle. In a particularly preferred embodiment, the present invention is predicted on the beacon ligand being clone 31/pPC86, which has strong homology to mouse clk4.

TABLE 5

Sequence data summary

Clone	Sequence
Clone 12/pPC86 cDNA	Partial sequence - 100% homologous to HSPB2
Clone 16/pPC86 cDNA	Partial sequence - 100% homologous to HSPB2
Clone 31/pPC86 cDNA	Partial sequence shows strong homology to mouse cdc2/CDC28-like protein kinase 4

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

BIBLIOGRAPHY

Barnett *et al.* (1994a) *Diabetologia* **37**:671-676.

Barnett *et al.*(1994b) *Int J Obesity* **18**:789-794.

Barnett *et al.* (1995) *Diabetes Nutr Metab* **8**:42-47.

Bennett, S.A. and Magnus, P. (1994) *Med J Aust* **161**:519-527.

Bouchard, C. The genetics of obesity. Boca Raton: CBC Press, 1994.

Ciechanover, A. and Schwartz, A.L. (1994) *FASEB J* **8**:182-191.

Collier *et al.* (1997a) *Ann New York Acad Sci* **827**:50-63.

Collier *et al.* (1997b) *Exp Clin Endocrinol Diabetes* **105**:36-37.

DeFronzo, R.A. (1988) *Diabetes* **37**:667-688.

Douillard and Hoffman, Basic Facts about Hybridomas, in *Compendium of Immunology* Vol. II, ed. by Schwartz, 1981.

Kohler and Milstein, (1975) *Nature* **256**:495-499.

Kohler and Milstein (1976) *European Journal of Immunology* **6**:511-519.

Kopelman *et al.* (1994) *Int J Obesity* **18**:188-191.

Leibowitz, S.F. (1985) *Fed Proc* **45**:1396-1403.

National Health and Medical Research Council (1996) Acting on Australia's weight: A strategy for the prevention of overweight and obesity. Canberra: National Health and Medical Research Council.

Risk Factor Prevalence Study Management Committee. Risk Factor Prevalence Study: Survey No. 3 1989.Canberra: National Heart Foundation of Australia and Australian Institute of Health, 1990.

Ravussin, E. (1995) *Metabolism* **44(Suppl 3)**:12-14.

Shafrir, E. and Gutman, A. (1993) *J Basic Clin Physiol Pharm* **4**:83-99.

Stellar, E. (1954) *Psychol Rev* **61**:5-22.

Walder *et al.* (1997a) *Obesity Res* **5**:193-200.

Walder *et al.*(1997b) *Int. J. Obesity* **22**:1-7, 1998.

Waters, A-M and Bennett, S. Risk Factors for cardiovascular disease: A summary of Australian data.Canberra: Australian Institute of Health and Welfare, 1995.

Zhang *et al.* (1994) *Nature* **372**:425-432.

Zimmet, P.Z. (1992) *Diabetes Care* **15(2)**:232-247.

CLAIMS

1. A ligand of a protein or a derivative, homologue, analogue or mimetic of said protein which protein is produced in larger amounts in hypothalamus tissue of obese animals compared to lean animals.

2. A ligand according to Claim 1 wherein the protein is encoded by a nucleotide sequence substantially as set forth in <400>1 or <400>4, a nucleotide sequence having at least 50% similarity after optimal alignment to <400>1 or <400>4 or a nucleotide sequence which is capable of hybridizing to <400>1 or <400>4 under low stringency conditions.

3. A ligand according to Claim 1 or 2 wherein the protein comprises an amino acid sequence substantially as set forth in <400>2 or <400>5 or an amino acid sequence having at least about 50% similarity after optimal alignment to <400>2 or <400>5.

4. A ligand according to any one of Claims 1 to 3 wherein protein is encoded by the nucleotide sequence substantially as set forth in <400>6 or <400>7 or a nucleotide sequence having at least 50% similarity after optimal alignment to <400>6 or <400>7 or a nucleotide sequence capable of hybridizing to <400>6 or <400>7 under low stringency conditions.

5. A ligand according to any one of Claims 1 to 3 wherein the ligand is encoded by the nucleotide sequence substantially as set forth in Figure 2 or Figure 3 or a nucleotide sequence having at leat about 50% similarity after optimal alignment to a nucleotide sequence in Figure 2 or Figure 3 or a nucleotide sequence capable of hybridizing to a nucleotide sequence in Figure 2 or Figure 3 under low stringency conditions.

6. A ligand according to Claim 1 wherein the ligand is a heat shock protein or a cdc-like protein.

7. A ligand according to Claim 6 wherein the ligand is a cdc-like kinase.

8. A method of identifying a ligand of the protein beacon or its derivatives, said method comprising introducing a first genetic construct in a yeast strain, said genetic construct comprising a nucleotide sequence encoding all or part of beacon fused to a nucleotide sequence encoding one of a DNA binding (DB) domain or an activation domain (AD) and introducing a second genetic construct into said yeast comprising a cDNA, said second genetic construct comprising elements of a cDNA library fused to a nucleotide sequence encoding the other of a DB domain or AD domain and selecting yeast cells which comprise both genetic constructs and in which a reporter gene has been subjected to two-hybrid dependent transcription.

9. A method according to Claim 8 wherein the ligand is encoded by the nucleotide sequence substantially as set forth in <400>6 or <400>7 or a nucleotide sequence having at least 50% similarity after optimal alignment to <400>6 or <400>7 or a nucleotide sequence capable of hybridizing to <400>6 or <400>7 under low stringency conditions.

10. A method according to Claim 8 wherein the ligand is encoded by the nucleotide sequence substantially as set forth in Figure 2 or Figure 3 or a nucleotide sequence having at leat about 50% similarity after optimal alignment to a nucleotide sequence in Figure 2 or Figure 3 or a nucleotide sequence capable of hybridizing to a nucleotide sequence in Figure 2 or Figure 3 under low stringency conditions.

11. A ligand according to Claim 8 wherein the ligand is a heat shock protein or a cdc-like protein.

12. A ligand according to Claim 11 wherein the ligand is a cdc-like kinase.

13. A ligand identified by the method of Claim 8.

14. A method for modulating expression of *beacon* ligand in a mammal, said mammal comprising contacting the *beacon* ligand gene with an effective amount of a modulator of *beacon* ligand expression for a time and under conditions or otherwise modulate expression of *beacon* ligand.

15. A method of modulating activity of beacon in a mammal, said method comprising administering to said mammal a modulating effective amount of a soluble *beacon* ligand or a derivative thereof for a time and under conditions sufficient to increase or decrease beacon activity.

16. A composition comprising a soluble form of beacon ligand or a modulator of *beacon* ligand expression and one or more pharmaceutically acceptable carriers and/or diluents.

17. A composition according to Claim 16 further comprising a leptin, a modulator of leptin activity or a modulator of *ob* expression.

```
                                5'    GTTCCAGGAGATTACAGCTCCAGCCACA
          3'   ATGTTCAACACAGCAGCCATCCAAGGTCCTCTAATGTCGAGGTCGGTGT

                                10                                  20
 M   I   E   V   V   C   N   D   R   L   G   K   K   V   R   V   K   C   N   T
ATGATTGAGGTGGTTTGCAACGACCGTCTAGGAAAGAAAGTCCGCGTTAAGTGCAACACC
TACTAACTCCACCAAACGTTGCTGGCAGATCCTTTCTTTCAGGCGCAATTCACGTTGTGG

                                30                                  40
 D   D   T   I   G   D   L   K   K   L   I   A   A   Q   T   G   T   R   W   N
GATGACACCATCGGGGACTTGAAGAAACTGATAGCGGCCCAAACTGGCACTCGTTGGAAT
CTACTGTGGTAGCCCCTGAACTTCTTTGACTATCGCCGGGTTTGACCGTGAGCAACCTTA

                                50                                  60
 K   I   V   L   K   K   W   Y   T   I   F   K   D   H   V   S   L   G   D   Y
AAGATCGTTCTTAAAAAGTGGTACACGATTTTTAAGGACCATGTATCTCTGGGAGATTAT
TTCTAGCAAGAATTTTTCACCATGTGCTAAAAATTCCTGGTACATAGAGACCCTCTAATA

                                70                                  40
 E   I   H   D   G   M   N   L   E   L   Y   Y   Q STOP
GAAATCCACGATGGGATGAACCTGGAGCTTTATTACCAGTAGAGGGGAATTCCTCCACC
CTTTAGGTGCTACCCTACTTGGACCTCGAAATAATGGTCATCTCCCCTTAAGGAGGTGG


TTGCCCAACCTTGCTTTCCTCTCCCATGGCTCATTTAACACTGTTGTAGATGCTCATTTTT
AACGGGTTGGAACGAAAGGAGAGGGTACCGAGTAAATTGTGACAACATCTACGAGTAAAAA


AACAATTCACATGAATAAAAACTTTGATGCTGCAAAAAAAAA   3'
TTGTTAAGTGTACT   5'
```

Figure 1a

• • • pPC86 Clone 31 Sequence • • •

(Human Homologue of Mouse Clk4)

1323 BPS DNA

→ Upstream sequence to be determined

```
   1  ATTATCACAG AGACATTGAA AGCGGGTATC GAATCCACTG CAGTAAATCT TCAGTCCGCA
  61  GCAGGAGAAG CAGTCCTAAA AGGAAGCGCA ATAGACACTG TTCAAGTCAT CAGTCACGTT
 121  CGAAGAGCCA CCGAAGGAAA AGATCCAGGA GTATAGAGGA TGATGAGGAG GGTCACCTGA
 181  TCTGTCAAAG TGGAGACGTT CTAAGAGCAA GATATGAAAT CGTGGACACT TTGGGTGAAG
 241  GAGCCTTTGG CAAAGTTGTA GAGTGCATTG ATCATGGCAT GGATGGCATG CATGTAGCAG
 301  TGAAAATCGT AAAAAATGTA GGCCGTTACC GTGAAGCAGC TCGTTCAGAA ATCCAAGTAT
 361  TAGAGCACTT AAATAGTACT GATCCCAATA GTGTCTTCCG ATGTGTCCAG ATGCTAGAAT
 421  GGTTTGATCA TCATGGTCAT GTT
```

Intervening sequence to be determined

```
   1  TTCAAATTGA CCACATCAGG CAGATGGCGT ATCAGATCTG CCAGTCAATA AATTTTTTAC
  61  ATCATAATAA ATTAACCCAT ACAGATCTGA AGCCTGAAAA TATTTTGTTT GTGAAGTCTG
 121  ACTATGTAGT CAAATATAAT TCTAAAATGA AACGTGATGA ACGCACACTG AAAAACACAG
 181  ATATCAAAGT TGTTGACTTT GGAAGTGCAA CGTATGATGA TGAACATCAC AGTACTTTGG
 241  TGTCTACCCG GCACTACAGA GCTCCCGAGG TCATTTTGGC TTTAGGTTGG TCTCAGCCTT
 301  GTGATGTTTG GAGCATAGGT TGCATTCTTA TTGAATATTA CCTTGGTTTC ACAGTCTTTC
 361  AGACTCATGA TAGTAAAGAG CACCTGGCAA TGATGGAACG AATATTAGGA CCCATACCAC
 421  AACACATGAT TCAGAAAACA AGAAAACGCA AGTATTTTCA CCATAACCAG CTAGATTGGG
 481  ATGAACACAG TTCTGCTGGT AGATATGTTA GGAGACGCTG CAAACCGTTG AAGGAATTTA
 541  TGCTTTGTCA TGATGAAGAA CATGAGAAAC TGTTTGACCT GGTTCGAAGA ATGTTAGAAT
 601  ATGATCCAAC TCAAAGAATT ACCTTGGATG AAGCATTGCA GCATCCTTTC TTTGACTTAT
 661  TAAAAAAGAA ATGAAATGGG AATCAGTGGT CTTACTATAT ACTTCTCTAG AAGAGATTAC
 721  TTAAGACTGT GTCAGTCAAC TAAACATTCT AATATTTTTG TAAACATTAA ATTATTTTGT
 781  ACAGTTAAGT GTAAATATTG TATGTTTTGT ATCAATAGCA TAATTAACTT GTTAAGCAAG
 841  TATGGTCTTG ATAATGCATT AGAAAAATTA AAATTAATTT TTCTTTTTGA AATTACCATT
 901  TTTAAATACC TTTGAAATAT CCTTTGTGTC CAGTGATAAA TGTGATTGAT CTTGCCTTTT
 961  GTACATGGAG GTCACCTCTG AAGTGATTTT TTTTGAGTAA AAGGAAATCT TGACTACTTT
1021  ATATTCTTAA AGGAATATTC TTTATATACT TCAAATTTAG AACTTAACTT TAAAAGTTTT
1081  TCTTCTGTAA TTGTTGAACG GGTGATTATT ATTAACTCTA GATAAGCAGG TACTAGAAAC
1141  CAAAACTCAG AAAATGTTTA CTGTTAGAAT TCTATTAAAT TTTAAGTGTT GTATTCTTTT
1201  TCATTGGGTG ATGTCAGGGT GATAACCAGA CATTCATGGA AAGGCATGCA GTTTGTCCAT
1261  TGTGACAGTT TGTTTAATAA AACCACATAC ACCCTTTAAA AAAAAAAAAA AAAAAAAAAA
1321  AAA
```

Figure 2

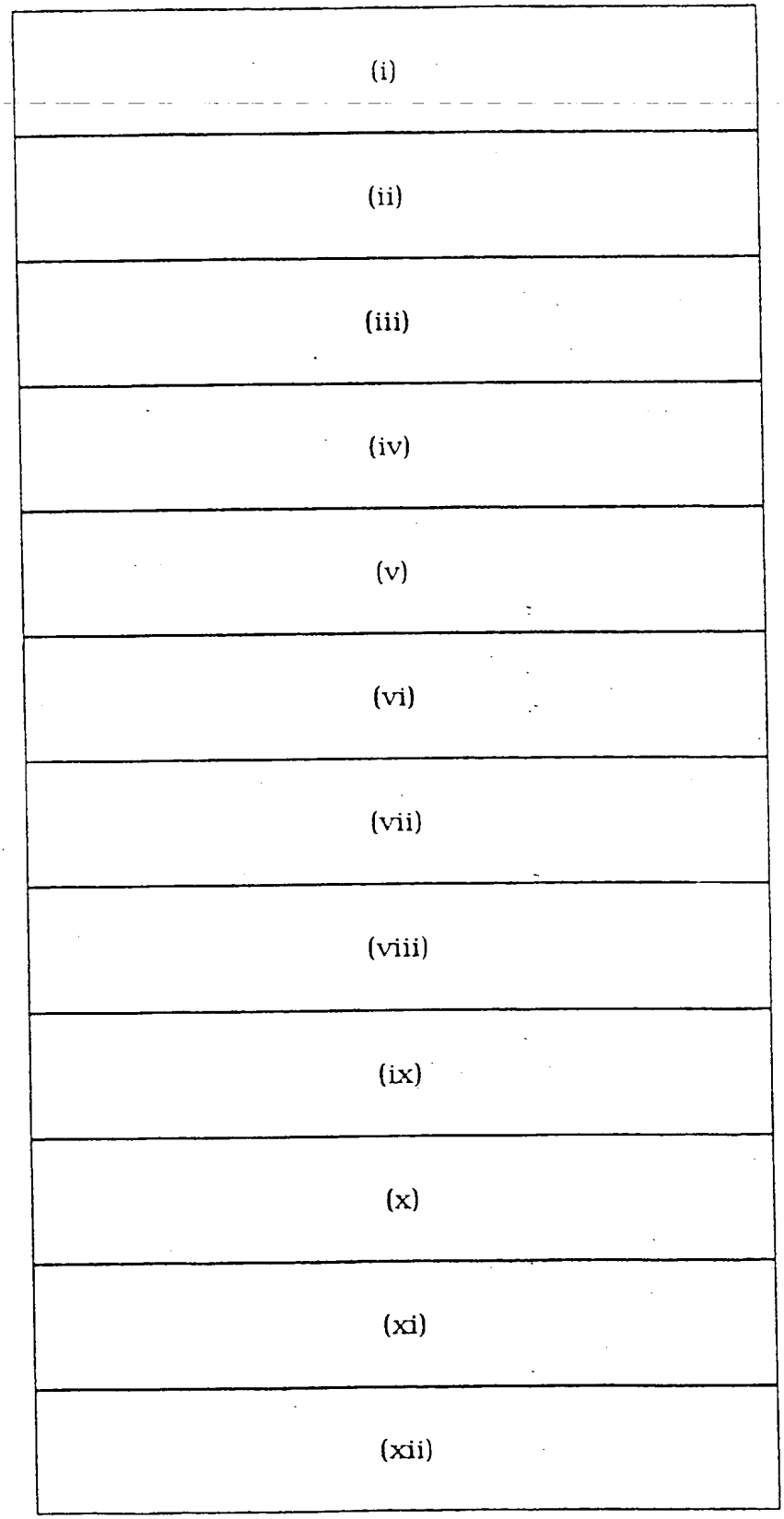


Figure 3

```
ATG ATC GAG GTT GTT TGC AAC GAC CGT CTG GGG AAA AAG GTC CNC GTT      48
Met Ile Glu Val Val Cys Asn Asp Arg Leu Gly Lys Lys Val Xaa Val
 1               5                  10                  15

AAA TGC AAC ACG GAT GAT ACC ATC GGG GAC CTT AAG AAG CTG ATT GCA      96
Lys Cys Asn Thr Asp Asp Thr Ile Gly Asp Leu Lys Lys Leu Ile Ala
            20                  25                  30

GCC TAA                                                             102
Ala *
```

Figure 1b

Partial Sequence Alignment

PPC86-31 and Mouse Clk4

Reference molecule: 31FSEQ.TXT 1 - 443 (443 bps) Homology

Sequence 2: CLK4M.TXT 341 - 783 (443 bps) 92%

Parameters set: Mismatch = 2; Open Gap = 0; Extend Gap = 2

```
31FSEQ.TXT    (   1)  ATTATCACAGAGACATTGAAAGCGGGTATCGAATCCACTGCAGTAAATCT
CLK4M.TXT     ( 341)  ....C..T.....G.........ACT..C..G.....T..........C
31FSEQ.TXT    (  51)  TCAGTCCGCAGCAGGAGAAGCAGTCCTAAAAGGAAGCGCAATAGACACTG
CLK4M.TXT     ( 391)  .....A.G.....................C.....G..A.....T.....C...
31FSEQ.TXT    ( 101)  TTCAAGTCATCAGTCACGTTCGAAGAGCCACCGAAGGAAAAGATCCAGGA
CLK4M.TXT     ( 441)  .G.....G.A.....................................
31FSEQ.TXT    ( 151)  GTATAGAGGATGATGAGGAGGGTCACCTGATCTGTCAAAGTGGAGACGTT
CLK4M.TXT     ( 491)  ....................................................
31FSEQ.TXT    ( 201)  CTAAGAGCAAGATATGAAATCGTGGACACTTTGGGTGAAGGAGCCTTTGG
CLK4M.TXT     ( 541)  ..................A.................................
31FSEQ.TXT    ( 251)  CAAAGTTGTAGAGTGCATTGATCATGGCATGGATGGCATGCATGTAGCAG
CLK4M.TXT     ( 591)  ..................C.............T.A.................
31FSEQ.TXT    ( 301)  TGAAAATCGTAAAAAATGTAGGCCGTTACCGTGAAGCAGCTCGTTCAGAA
CLK4M.TXT     ( 641)  .........T.....................A.....G..G.....T....
31FSEQ.TXT    ( 351)  ATCCAAGTATTAGAGCACTTAAATAGTACTGATCCCAATAGTGTCTTCCG
CLK4M.TXT     ( 691)  ..........G.....G..C..C.....C.....C..................
31FSEQ.TXT    ( 401)  ATGTGTCCAGATGCTAGAATGGTTTGATCATCATGGTCATGTT
CLK4M.TXT     ( 741)  ...C.....G.....................................
```

Figure 3(i)

Multiple Sequence Alignment

pPC86-31

Reference molecule:	31SEQ.TXT	1 - 1323	(1323 bps)	Homology
Sequence 2:	CLK4M.TXT	1 - 1549	(1549 bps)	47%
Sequence 3:	STYMA.TXT	1 - 1740	(1740 bps)	51%
Sequence 4:	CLK1H.TXT	1 - 1834	(1834 bps)	53%

Parameters set: Mismatch = 2; Open Gap = 4; Extend Gap = 1

```
31SEQ.TXT      (   1)  --------------------------------------------------
CLK4M.TXT      (   1)  AAAGAGACGCAGCGGCTGGAGAGGAACGACGGCGGTTTGGCGACATTTCT
STYMA.TXT      (   1)  ATCGTCGTAATCGTTTGCAGACTTCTCGCCGTCGCCTTGTAAGCTTTGTC
CLK1H.TXT      (   1)  ATTTTTAGATAATCATTAAAGACCACAGAAAATGTAACAGATCCTACTC-

31SEQ.TXT      (   1)  --------------------------------------------------
CLK4M.TXT      (  51)  GCCCAAAAGGCCGCTTGCTTTTGCGGAGATGCGGCATTCCAAACGAACTC
STYMA.TXT      (  51)  TTCGCCTTGCAAGCTTTGTCTTCAGGGTTGGAAAGATGAGACATTCAAAG
CLK1H.TXT      (  50)  --------------------------------------------------

31SEQ.TXT      (   1)  --------------------------------------------------
CLK4M.TXT      ( 101)  ACTGTCCTGATTGGGATAGTAGAGAAAGCTGGGGCCATGAAAGCTACAGT
STYMA.TXT      ( 101)  AGAACTTACTGTCCTGACTGGGATGAAAGAGACTGGGATTATGGAACATG
CLK1H.TXT      (  50)  --------------------------------------------------

31SEQ.TXT      (   1)  --------------------------------------------------
CLK4M.TXT      ( 151)  GGAAGTCACAAACGCAAGAGAAGGTCTCACAGCAGTACTCAGGAGAACAG
STYMA.TXT      ( 151)  GAGAAGCAGCAGCAGTCACAAAAGAAAGAAGAGATCACATAGCAGCGCCC
CLK1H.TXT      (  50)  --------------------------------------------------
```

Figure 3(ii)

```
31SEQ.TXT   (   1)  --------------------------------------------------
CLK4M.TXT   ( 201)  GCACTGTAAACCACATCATCAGTTTAAAGACTCGGATTGTCACTATTTAG
STYMA.TXT   ( 201)  GTGAGCAAAAGCGCTGCAGGTACGATCACTCCAAAACGACAGACAGCTAT
CLK1H.TXT   (  50)  --------------------------------------------------

31SEQ.TXT   (   1)  --------------------------------------------------
CLK4M.TXT   ( 251)  AAGCAAGATGCTTGAATGAGAGAGATTATCGGGACCGGAGATACATTGAT
STYMA.TXT   ( 251)  TATCTGGAAAGCAGATCCATAAATGAGAAAGCTTATCATAGTCGACGCTA
CLK1H.TXT   (  50)  --------------------------------------------------

31SEQ.TXT   (   1)  --------------------------------------------------
CLK4M.TXT   ( 301)  GAATACAGAAATGACTACTGCGAAGGATATGTTCCAAGACATTACCATAG
STYMA.TXT   ( 301)  TGTTGATGAATACAGGAATGACTACATGGGCTACGAGCCAGGGCATCCCT
CLK1H.TXT   (  50)  --------------------------------------------------

31SEQ.TXT   (   1)  --------------------------------------------------
CLK4M.TXT   ( 351)  AGACGTTGAAAGCACTTACCGGATCCATTGCAGTAAATCCTCAGTCAGGA
STYMA.TXT   ( 351)  ATGGAGAACCTGGAAGCAGATACCAGATGCATAGTAGCAAGTCCTCTGGT
CLK1H.TXT   (  50)  --------------------------------------------------

31SEQ.TXT   (   1)  --------------------------------------------------
CLK4M.TXT   ( 401)  GCAGGAGAAGCAGCCCTAAGAGAAAGCGTAATAGACCCTGTGCAAGTCAT
STYMA.TXT   ( 401)  AGGAGTGGAAGAAGCAGTTACAAAAGTAAACACAGGAGTCGCCACCACAC
CLK1H.TXT   (  50)  --------------------------------------------------
```

Figure 3iii

```
31SEQ.TXT    (    1)  ----------------------------------------------------
CLK4M.TXT    (  451)  CAGTCGCATTCGAAGAGCCACCGAAGGAAAAGATCCAGGAGTATAGAGGA
STYMA.TXT    (  451)  ATCGCAGCACCATTCACACGGGAAGAGTCACCGAAGGAAAAGATCGAGGA
CLK1H.TXT    (   50)  ----------------------------------------------------

31SEQ.TXT    (    1)  ----------------------------------------------------
CLK4M.TXT    (  501)  TGATGAGGAGGGTCACCTGATCTGTCAAAGTGGAGACGTTCTAAGAGCAA
STYMA.TXT    (  501)  GTGTAGAGGATGATGAGGAGGGTCACCTGATCTGTCAGAGTGGAGACGTA
CLK1H.TXT    (   50)  ----------------------------------------------------

31SEQ.TXT    (    1)  ----------------------------------------------------
CLK4M.TXT    (  551)  GATATGAAATCGTGGACACTTTAGGTGAAGGAGCCTTTGGCAAAGTTGTA
STYMA.TXT    (  551)  CTAAGTGCAAGATATGAAATTGTTGATACTTTAGGTGAAGGTGCTTTCGG
CLK1H.TXT    (   50)  ----------------------------------------------------

31SEQ.TXT    (    1)  ----------------------------------------------------
CLK4M.TXT    (  601)  GAGTGCATTGATCACGGCATGGATGGCTTACATGTAGCAGTGAAAATTGT
STYMA.TXT    (  601)  AAAAGTGGTGGAATGCATCGATCATAAAGTGGGAGGTAGACGTGTAGCAG
CLK1H.TXT    (   50)  ----------------------------------------------------

31SEQ.TXT    (    1)  ----------------------------------------------------
CLK4M.TXT    (  651)  AAAAAATGTAGGACGTTACCGGGAGGCAGCTCGTTCTGAAATCCAAGTAT
STYMA.TXT    (  651)  TAAAAATAGTTAAAAATGTGGATAGATACTGTGAAGCTGCTCAATCGGAA
CLK1H.TXT    (   50)  ----------------------------------------------------

31SEQ.TXT    (    1)  ----------------------------------------------------
CLK4M.TXT    (  701)  TGGAGCACTTGAACAGCACTGACCCCAACAGTGTCTTCCGATGCGTCCAG
STYMA.TXT    (  701)  ATACAAGTTTTGGAACACTTGAATACAACAGACCCCCATAGTACTTTCCG
CLK1H.TXT    (   50)  ----------------------------------------------------
```

Figure 3(iv)

```
31SEQ.TXT    (    1) ----------------------------------------------------
CLK4M.TXT    (  751) ATGCTAGAGTGGTTTGATCATCATGGTCATGTTTGTATTGTGTTTGAGCT
STYMA.TXT    (  751) TTGTGTCCAGATGTTGGAGTGGTTTGAGCATCGAGGTCACATTTGCATTG
CLK1H.TXT    (   50) ----------------------------------------------------

31SEQ.TXT    (    1) ----------------------------------------------------
CLK4M.TXT    (  801) GCTGGGACTTAGTACCTATGATTTTATTAAAGAAAATAGTTTTCTGCCAT
STYMA.TXT    (  801) TGTTTGAACTTCTGGGGCTTAGTACTTATGATTTCATTAAGGAAAACAGT
CLK1H.TXT    (   50) ----------------------------------------------------

31SEQ.TXT    (    1) ----------TTCAAA----------------------------------
CLK4M.TXT    (  851) ----------.....----------------------------------
STYMA.TXT    (  851) TTTCTGCCGT...G.----------------------------------
CLK1H.TXT    (   50) ----------.....ATAATTGCTATTCAGTATTAAACGAGCAGTCAG

31SEQ.TXT    (    7) ----------------------------------------------------
CLK4M.TXT    (  857) ----------------------------------------------------
STYMA.TXT    (  867) ----------------------------------------------------
CLK1H.TXT    (   90) CTGCGTGATTCCCGTGATTGCGTTACAAGCTTTGTCTCCTTCGACTTGGA

31SEQ.TXT    (    7) ----------------------------------------------------
CLK4M.TXT    (  857) ----------------------------------------------------
STYMA.TXT    (  867) ----------------------------------------------------
CLK1H.TXT    (  140) GTCTTTGTCCAGGACGATGAGACACTCAAAGAGAACTTACTGTCCTGATT

31SEQ.TXT    (    7) ----------------------------------------------------
CLK4M.TXT    (  857) ----------------------------------------------------
STYMA.TXT    (  867) ----------------------------------------------------
CLK1H.TXT    (  190) GGGATGACAAGGATTGGGATTATGGAAAATGGAGGAGCAGCAGTCAT
```

Figure 3(v)

```
31SEQ.TXT    (   7)  ----------------------------------------
CLK4M.TXT    ( 857)  ----------------------------------------
STYMA.TXT    ( 867)  ----------------------------------------
CLK1H.TXT    ( 240)  AAAAGAAGGAAGAGATCACATAGCAGTGCCCAGGAGAACAAGCGCTGCAA

31SEQ.TXT    (   7)  ----------------------------------------
CLK4M.TXT    ( 857)  ----------------------------------------
STYMA.TXT    ( 867)  ----------------------------------------
CLK1H.TXT    ( 290)  ATACAATCACTCTAAAATGTGTGATAGCCATTATTTGGAAAGCAGGTCTA

31SEQ.TXT    (   7)  ----------------------------------------
CLK4M.TXT    ( 857)  ----------------------------------------
STYMA.TXT    ( 867)  ----------------------------------------
CLK1H.TXT    ( 340)  TAAATGAGAAAGATTATCATAGTCGACGCTACATTGATGAGTACAGAAAT

31SEQ.TXT    (   7)  ----------------------------------------
CLK4M.TXT    ( 857)  ----------------------------------------
STYMA.TXT    ( 867)  ----------------------------------------
CLK1H.TXT    ( 390)  GACTACACTCAAGGATGTGAACCTGGACATCGCCAAAGAGACCATGAAAG

31SEQ.TXT    (   7)  ----------------------------------------
CLK4M.TXT    ( 857)  ----------------------------------------
STYMA.TXT    ( 867)  ----------------------------------------
CLK1H.TXT    ( 440)  CCGGTATCAGAACCATAGTAGCAAGTCTTCTGGTAGAAGTGGAAGAAGTA

31SEQ.TXT    (   7)  ----------TTGACCACA----------------------
CLK4M.TXT    ( 857)  ----------....T.....----------------------
STYMA.TXT    ( 867)  ----------.G..T..T.----------------------
CLK1H.TXT    ( 490)  GTTATAAAAGCAAACACAGGA..C.....GTACTTCACATCGTCGTTCA
```

Figure 3(vi)

```
31SEQ.TXT  (  16)  ----------------------------------------
CLK4M.TXT  (  866)  ----------------------------------------
STYMA.TXT  (  876)  ----------------------------------------
CLK1H.TXT  (  540)  CATGGGAAGAGTCACCGAAGGAAAAGAACCAGGAGTGTAGAGGATGATGA

31SEQ.TXT  (  16)  ----------  ----------
CLK4M.TXT  (  866)  ----------------------------------------
STYMA.TXT  (  876)  ----------------------------------------
CLK1H.TXT  (  590)  GGAGGGTCACCTGATCTGTCAGAGTGGAGACGTACTAAGTGCAAGATATG

31SEQ.TXT  (  16)  ----------------------------------------
CLK4M.TXT  (  866)  ----------------------------------------
STYMA.TXT  (  876)  ----------------------------------------
CLK1H.TXT  (  640)  AAATTGTTGATACTTTAGGTGAAGGAGCTTTTGGAAAAGTTGTGGAGTGC

31SEQ.TXT  (  16)  ----------------------------------------
CLK4M.TXT  (  866)  ----------------------------------------
STYMA.TXT  (  876)  ----------------------------------------
CLK1H.TXT  (  690)  ATCGATCATAAAGCGGGAGGTAGACATGTAGCAGTAAAAATAGTTAAAAA

31SEQ.TXT  (  16)  ----------------------------------------
CLK4M.TXT  (  866)  ----------------------------------------
STYMA.TXT  (  876)  ----------------------------------------
CLK1H.TXT  (  740)  TGTGGATAGATACTGTGAAGCTGCTCGCTCAGAAATACAAGTTCTGGAAC

31SEQ.TXT  (  16)  ----------------------------------------
CLK4M.TXT  (  866)  ----------------------------------------
STYMA.TXT  (  876)  ----------------------------------------
CLK1H.TXT  (  790)  ATCTGAATACAACAGACCCCAACAGTACTTTCCGCTGTGTCCAGATGTTG
```

Figure 3(vii)

```
31SEQ.TXT    (  16)  ----------------------------------------
CLK4M.TXT    (  866) ----------------------------------------
STYMA.TXT    (  876) ----------------------------------------
CLK1H.TXT    (  840) GAATGGTTTGAGCATCATGGTCACATTTGCATTGTTTTTGAACTATTGGG

31SEQ.TXT    (  16)  ----------------------------------------
CLK4M.TXT    (  866) ----------------------------------------
STYMA.TXT    (  876) ----------------------------------------
CLK1H.TXT    (  890) ACTTAGTACTTACGACTTCATTAAAGAAAATGGTTTTCTACCATTTCGAC

31SEQ.TXT    (  16)  ---------TCAGGCAGATGGCGTATCAGATCTGCCAGTCAATAAATTTT
CLK4M.TXT    (  866) ---------.....A.....T.....T.....
STYMA.TXT    (  876) ---------.....A.....A...A...A.A..TG....C...
CLK1H.TXT    (  940) TGGATCATA....AA.....A.....A....A....TG.G.....

31SEQ.TXT    (  57)  TTACATCATAATAAATTAACCCATACAGATCTGAAGCCTGAAAATATTTT
CLK4M.TXT    (  907) .....A..C..G..C..A..A.....C..C.
STYMA.TXT    (  917) ..G...AG.....G..T.....CT.....C..C.
CLK1H.TXT    (  990) ..G..CAG.....G..G..T..C.....CT.A.....C..C..

31SEQ.TXT    (  107) GTTTGTGAAGTCTGACTATGTAGTCAAATATAATTCTAAAATGAAACGTG
CLK4M.TXT    (  957) A.....C.....A.
STYMA.TXT    (  967) A.....CAC..AGGCT.....C.C.....
CLK1H.TXT    (1040)  A.....C.....CAC..AGGCG.....C.C.....A.....

31SEQ.TXT    (  157) ATGAACGCACACTGAAAAACACAGATATCAAAGTTGTTGACTTTGGAAGT
CLK4M.TXT    (1007)  ....G.....T.....T.....
STYMA.TXT    (1017)  .....T..TA.AGT...TC.....T.....G..G.....
CLK1H.TXT    (1090)  .....CT.A.T...TC.....T.....A.....T...
```

Figure 3(viii)

```
31SEQ.TXT  ( 207)  GCAACGTATGATGAACATCACAGTACTTTGGTGTCTACCCGGCACTA
CLK4M.TXT  (1057)  .....A.....C..C.........T.....................C..AA.....
STYMA.TXT  (1067)  .....A.....................C.....C..A.....A.....AA.A..T..
CLK1H.TXT  (1140)  .....A.....C.....................A.....A.....AA.A..T..

31SEQ.TXT  ( 257)  CAGAGCTCCCGAGGTCATTTTGGCTTTAGGTTGGTCTCAGCCTTGTGATG
CLK4M.TXT  (1107)  ...G.....A.....................C.....................
STYMA.TXT  (1117)  T.....A..G..A..T.....A..CC.C..G.....A.....A.....
CLK1H.TXT  (1190)  T.....A..T..A..T.....A..CC.....G.....C..A..A.....

31SEQ.TXT  ( 307)  TTTGGAGCATAGGTTGCATTCTTATTGAATATTACCTTGGTTTCACAGTC
CLK4M.TXT  (1157)  .....C.....G..C.....G.....
STYMA.TXT  (1167)  .C.....A..T.....C..G.....T.....A..T.....T
CLK1H.TXT  (1240)  .C.....A.....C..T.....G..T..C..A

31SEQ.TXT  ( 357)  TTTCAGACTCATGATAGTAAAGAGCACCTGGCAATGATGGAACGAATATT
CLK4M.TXT  (1207)  .....C..C.....G..G..C..
STYMA.TXT  (1217)  ...TC.....C.GG..A..TT.A.....A.G..TC.
CLK1H.TXT  (1290)  ....CA..A..C.....G.....TT.A.....A.G..TC.

31SEQ.TXT  ( 407)  AGGACCCATACCACACAACACATGATTCAGAAAACAAGAAAACGCAAGTATT
CLK4M.TXT  (1257)  .....C...GC...T.....C.....G.....G.....
STYMA.TXT  (1267)  T.....AC.....A.G.....A.....C..G.....GA....
CLK1H.TXT  (1340)  T.....TC.....A.....T.....A.....C..G.....T..A....

31SEQ.TXT  ( 457)  TTCACCATAACCAGCTAGATTGGGATGAACACAGTTCTGCTGGTAGATAT
CLK4M.TXT  (1307)  .C.....C..G..T.....A.....G.....
STYMA.TXT  (1317)  .C..T...G.T.GAT.....C.....
CLK1H.TXT  (1390)  .....CG.T.GAT....C.....C..C.....
```

Figure 3(ix)

```
31SEQ.TXT   ( 507)  GTTAGGAGACGCTGCAAACCGTTGAAGGAATTTATGCTTTGTCATGATGA
CLK4M.TXT   (1357)  ..........G.....A..................C..
STYMA.TXT   (1367)  ...TCTC.G.....T.....TC.....G.....A.C...G.....C
CLK1H.TXT   (1440)  ...TCA...GC...T.....TC.....C...A.....T

31SEQ.TXT   ( 557)  AGAACATGAGAAACTGTTTGACCTGGTTCGAAGAATGTTAGAATATGATC
CLK4M.TXT   (1407)  ...G.....G.....G..G..G.....C.
STYMA.TXT   (1417)  C.........CTT..C.....CA..G.G.A.....G..G.....
CLK1H.TXT   (1490)  T.........CGT..C.....CA...AG.A.....G..G.....

31SEQ.TXT   ( 607)  CAACTCAAAGAATTACCTTGGATGAAGCATTGCAGCATCCTTTCTTTGAC
CLK4M.TXT   (1457)  ..G.GAG...G..C.....C.....
STYMA.TXT   (1467)  .CG.CA.....TC.CA.A.....CC.AA.....T..
CLK1H.TXT   (1540)  ..G.CA.....TC.CAGA.....C..AA.....

31SEQ.TXT   ( 657)  TTATTAAAAAAGAAATGAAATGGGAATCAGTGGTCTTACTATATACTTCT
CLK4M.TXT   (1507)  .........G.....-.G.....G....----------
STYMA.TXT   (1517)  CC.C.T.....----------.C..AC...A.T.ATAA.C.C.G.G..
CLK1H.TXT   (1590)  C.TC.G..G..A.----------.T.TAT..ATC.G.A.T.GG.C.GC...

31SEQ.TXT   ( 707)  CTAGAAGAGATTACTTA-AGACTGTGTCAGTCAACTAAACATTCTAATAT
CLK4M.TXT   (1536)  ----------
STYMA.TXT   (1555)  ..GA...GA..--.....C.....A....----------....GCT.
CLK1H.TXT   (1631)  ..C.....--.....C.....A....----------.....---

31SEQ.TXT   ( 756)  TTTTGTAAACATTAAATTATTTGTACAGTTAAGTGTAAATATTGTATGT
CLK4M.TXT   (1536)  ----------
STYMA.TXT   (1591)  ..-------A.....G.....----------
CLK1H.TXT   (1664)  -...T....TT....G.....----------.C
```

Figure 3(x)

```
31SEQ.TXT   ( 806) TTTGTATCAATAGCATAATTAACTTGTTAAGCAAGTATGGTCTTGATAAT
CLK4M.TXT   (1536) ------------------------------------------------
STYMA.TXT   (1609) ----------.....T.....----------------------------
CLK1H.TXT   (1693) ----------...A..C.....----------------------------

31SEQ.TXT   ( 856) GCATTAGAAAAATTAAAATTAATTTTTCTTTTTGAAATTACCATTTTTAA
CLK4M.TXT   (1536) ------------------------------------------------
STYMA.TXT   (1623) ----------...AC.T...A.G.....T.G..--G.T..A.....
CLK1H.TXT   (1708) ----------------------A.....T.T...G.....G.....--

31SEQ.TXT   ( 906) ATACCTTTGAAATATCCTTTGTGTCCAGTGATAAATGTGATTGATCTTGC
CLK4M.TXT   (1536) ------------------------------------------------
STYMA.TXT   (1656) ....A.----------------------------------------...
CLK1H.TXT   (1729) ------------------------------------------------

31SEQ.TXT   ( 956) CTTTTGTACATGGAGGTCACCTCTGAAGTGATTTTTTTTTGAGTAAAAGGA
CLK4M.TXT   (1536) ----------..C..C.G.----------------------------
STYMA.TXT   (1662) ------------------------------------------G.CC...CC.
CLK1H.TXT   (1729) ----------------------------.....G.....G.....-----

31SEQ.TXT   (1006) AATCTTGACTACTTTATATTCTTAAAGGAATATTCTTTATATACTTCAAA
CLK4M.TXT   (1545) ------------------------------------------------
STYMA.TXT   (1672) ..----.A..AT.....CAG.AA....T..A.TG.....----------T.
CLK1H.TXT   (1744) ----------..TGG...A.T.....----------.C...GC.A.GG

31SEQ.TXT   (1056) TTTAGAACTTAACTTTAAAAGTTTTTCTTCTGTAATTGTTGAACGGGTGA
CLK4M.TXT   (1545) ------------------------------------------------
STYMA.TXT   (1705) ...G.....----------.....T..G.G...A.....----------
CLK1H.TXT   (1771) .AAT.....----------A.C..T...A.....AA.----------...
```

Figure 3(xi)

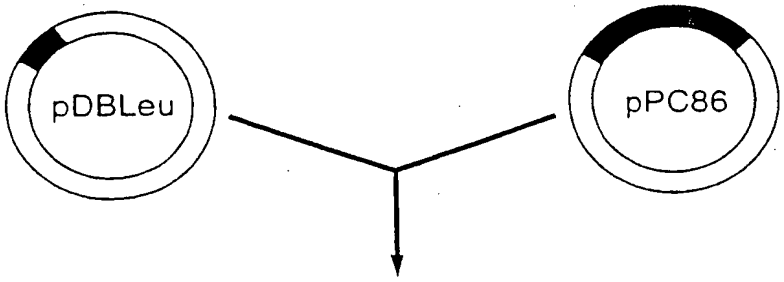
```
31SEQ.TXT  (1106)  TTATTATTAACTCTAGATAAGCAGGTACTAGAAACCAAAACTCAGAAAAT
CLK4M.TXT  (1545)  ----------------------------------------------------
STYMA.TXT  (1729)  ----------------------------------------------------...
CLK1H.TXT  (1804)  ..----------------------------------------T.T.....---

31SEQ.TXT  (1156)  GTTTACTGTTAGAATTCTATTAAATTTTAAGTGTTGTATTCTTTTTTCATT
CLK4M.TXT  (1545)  ----------------------------------------------------
STYMA.TXT  (1732)  ..----------------------------------------------------
CLK1H.TXT  (1815)  ----------.....----------------------------------------

31SEQ.TXT  (1206)  GGGTGATGTCAGGGTGATAACCAGACATTCATGGAAAGGCATGCAGTTTG
CLK4M.TXT  (1545)  ----------------------------------------------------
STYMA.TXT  (1734)  ----------------------------------------------------
CLK1H.TXT  (1822)  ----------------------------------------------------...

31SEQ.TXT  (1256)  TCCATTGTGACAGTTTGTTTAATAAAACCACATACACCCTTTAAAAAAAA
CLK4M.TXT  (1545)  ----------------------------..G.----------------------
STYMA.TXT  (1734)  ----------------------------------------------------
CLK1H.TXT  (1826)  .G.T.----------------------------------------------------

31SEQ.TXT  (1306)  AAAAAAAAAAAAAAAAAA
CLK4M.TXT  (1550)  ------------------
STYMA.TXT  (1734)  ----------.....
CLK1H.TXT  (1831)  ----------.TG.
```

Figure 3(xii)

Methodology

Clone Beacon into the vector pDBLeu

Human Brain cDNA library constructed in the vector pPC86



Transform both plasmids into the yeast strain MaV203

Select for cells containing both plasmids, and induce the *HIS3* reporter gene

Purify cells containing interacting proteins and test for induction of the 3 reporter genes

Isolate plasmid DNA from clones expressing the three reporter genes and perform a re-transformation assay to confirm phenotypes

Isolate plasmid DNA from positive clones and sequence unknown cDNA's

Confirm the interactions by independent methods such as co-immunoprecipitation and biological assays

Figure 4

SEQUENCE LISTING

<110> AUTOGEN PTY LTD

<120> A LIGAND

<130> 2269272/EJH

<140> Not yet assigned
<141> 2000-04-19

<150> PP9919/99
<151> 1999-04-23

<150> PQ6454/00
<151> 2000-03-24

<160> 7

<170> PatentIn Ver. 2.1

<210> 1
<211> 342
<212> DNA
<213> Israeli Sand Rat

<220>
<221> CDS
<222> (29)..(247)

<400> 1
gttccaggag attacagctc cagccaca atg att gag gtg gtt tgc aac gac 52
Met Ile Glu Val Val Cys Asn Asp
1 5

cgt cta gga aag aaa gtc cgc gtt aag tgc aac acc gat gac acc atc 100
Arg Leu Gly Lys Lys Val Arg Val Lys Cys Asn Thr Asp Asp Thr Ile
10 15 20

ggg gac ttg aag aaa ctg ata gcg gcc caa act ggc act cgt tgg aat 148

Gly Asp Leu Lys Lys Leu Ile Ala Ala Gln Thr Gly Thr Arg Trp Asn
25 30 35 40

aag atc gtt ctt aaa aag tgg tac acg att ttt aag gac cat gta tct 196
Lys Ile Val Leu Lys Lys Trp Tyr Thr Ile Phe Lys Asp His Val Ser
45 50 55

ctg gga gat tat gaa atc cac gat ggg atg aac ctg gag ctt tat tac 244
Leu Gly Asp Tyr Glu Ile His Asp Gly Met Asn Leu Glu Leu Tyr Tyr
60 65 70

cag tagaggggaa ttcctccacc ttgcccaacc ttgctttcct ctcccatggc 297
Gln

tcatttaaca ctgttgtaga tgctcatttt tttgttaagt gtact 342

<210> 2
<211> 73
<212> PRT
<213> Israeli Sand Rat

<400> 2
Met Ile Glu Val Val Cys Asn Asp Arg Leu Gly Lys Lys Val Arg Val
1 5 10 15

Lys Cys Asn Thr Asp Asp Thr Ile Gly Asp Leu Lys Lys Leu Ile Ala
20 25 30

Ala Gln Thr Gly Thr Arg Trp Asn Lys Ile Val Leu Lys Lys Trp Tyr
35 40 45

Thr Ile Phe Lys Asp His Val Ser Leu Gly Asp Tyr Glu Ile His Asp
50 55 60

Gly Met Asn Leu Glu Leu Tyr Tyr Gln
65 70

<210> 3

<211> 391
<212> DNA
<213> Israeli Sand Rat

<400> 3
atgttcaaca cagcagccat ccaaggtcct ctaatgtcga ggtcggtgtt actaactcca 60
ccaaacgttg ctggcagatc ctttctttca ggcgcaattc acgttgtggc tactgtggta 120
gcccctgaac ttctttgact atcgccgggt ttgaccgtga gcaaccttat tctagcaaga 180
atttttcacc atgtgctaaa aattcctggt acatagagac cctctaatac tttaggtgct 240
accctacttg gacctcgaaa taatggtcat ctcccctaa ggaggtggaa cgggttggaa 300
cgaaaggaga gggtaccgag taaattgtga caacatctac gagtaaaaaa acaattcaca 360
tgaataaaaa ctttgatgct gcaaaaaaaa a 391

<210> 4
<211> 2180
<212> DNA
<213> Human cells

<400> 4
accaatctga agtgggagcg accggggttc agcgctcggg tgaggagctg gtggcgtcgg 60
caggttcgag gcgattcgag gtgaggggat caagcggaga ggctcggagt cggagaaagc 120
tgtcgcgacc cagccaccca gggtctgggg tcggtgggag gtgaggccag ggagttttgt 180
ggcagggctt tcaggcggcg agaaggggcg gaggcgcggc tccccggggt tcgcggccct 240
gggacagggg acttggggcc tgggagtatt cgagtccggg gggttggggg cagaacggaa 300
ggctcagtaa cctggaattt tagggcctgg gactgggaga cttgtaggct ggggcttctg 360
ggcctgggag acgcctgaag ctctgacttt gctgcctccc acccaccccc cgctttgtgt 420
agctccagct aggatgatcg aggttgtttg caacgaccgt ctggggaaga aggtccgcgt 480
taaatgcaag tatccactgg cagccgagag gcagtggtac ccgcaggggt gcttggcgtg 540
aggcaggcaa ctcaatctgt gttgtcggat ggttttagtt aaacccggga gaggggggcgg 600
atggggtcct ggcagatggt attctgccct agaatgtcct cttcctcgtt ctacccgctt 660
ccatttgcct taactgctct gcgcccagca cggatgatac catcggggac cttaagaagc 720
tgattgcagc ccaaactggt acccgttgga acaagattgt cctgaagaag tggtgagtgc 780
agcggtagag ccactgggtg gactggacta ggccggaagg ttttggttgg gggagcgctg 840
caggcagccc tttgcttagg cttggctacc tcatttggcc tacagactga agagcctcct 900
tagccttatc tctgaaatgt ctctttttct taggtacacg atttttaagg accacgtgtc 960
tctgggggac tgtatccttt gtgtgacttt ttgaggaagc atctacatac ccagcctcgg 1020
ttatctgttc aaaactaaag tctgcatgag cttatgcata ttaagtgttt aacttaatac 1080
gtggcacatg gtgagcactt agaaaatggg agctaggtaa ttatttggtt ggggaggaga 1140
gtggttggtg aaatagtttg acttcaagtt caaaatattt attactgacc tccacctgag 1200
tgctggacag taggtcaggg tggggtgaga ctcaggggta tctattgtgc aaaaggagca 1260

gaaacttttt acaattttta ttttatttat ttatttttt gagacagtct ggctctgtcg 1320
cccaggctgg agtgcagtgg cacgatcttg acccactgca acctccacct ccc ggttca 1380
agcaattctg cctcagcttc ccaagtagct gggattacag gtgtgtgcca ccactccctg 1440
ctagtttttg tatttttagt agagatgagg ttccaccatg tatgccaggc tagtctcaaa 1500
ctcctggcct caagtgatcc tcccgcctcg gcctcccaaa gtgctgggat tatagcccgt 1560
gagccactgt gcctggccta aaaagaaaaa aaantttttt ttttttttt tgagatggag 1620
ttttgctctt gttgcccagg atggagtgca gtggtacaat cttggctcac tgcagcctct 1680
acctcccagg tttaagcgat tttcctgtct cagccttcca agtagctggg attacaggtg 1740
cccaccacca cacccagcta acttttttgta tttttagtag agacagggtt tcatcgtgtt 1800
ggccaggctg gtctcaaact cctgacgtca agtgagccac ctgcttcgtc ctctcaaagt 1860
gctgggatta caggtgtgag ccactgcacc cagccagaaa ttcttttgta aagttttggt 1920
tctctccaaa taaaaatggc ctggtccaaa atgggcttcc ttagccctgg gctgagacct 1980
caggccacct gacccggctg tgaatggatg agaggggtgg ggacggaagt cagagtcagg 2040
attccttaac acctttcctt cagatgaaat ccacgatggg atgaactgga gcttaatttc 2100
aatagatgag aatcctcatc ttcctgcccc gctttcctct cccatcctca tcccccacac 2160
tgggatagat gcttgtttgt 2180

<210> 5
<211> 102
<212> DNA
<213> human

<400> 5
atgatcgagg ttgtttgcaa cgaccgtctg gggaaaaagg tccncgttaa atgcaacacg 60
gatgatacca tcggggacct taagaagctg attgcagcct aa 102

<210> 6
<211> 443
<212> DNA
<213> human

<400> 6
attatcacag agacattgaa agcgggtatc gaatccactg cagtaaatct tcagtccgca 60
gcaggagaag cagtcctaaa aggaagcgca atagacactg ttcaagtcat cagtcacgtt 120
cgaagagcca ccgaaggaaa agatccagga gtatagagga tgatgaggag ggtcacctga 180
tctgtcaaag tggagacgtt ctaagagcaa gatatgaaat cgtggacact ttgggtgaag 240
gagcctttgg caaagttgta gagtgcattg atcatggcat ggatggcatg catgtagcag 300
tgaaaatcgt aaaaaatgta ggccgttacc gtgaagcagc tcgttcagaa atccaagtat 360
tagagcactt aaatagtact gatcccaata gtgtcttccg atgtgtccag atgctagaat 420

ggtttgatca tcatggtcat gtt 443

<210> 7
<211> 1323
<212> DNA
<213> human

<400> 7
ttcaaattga ccacatcagg cagatggcgt atcagatctg ccagtcaata aatttttac 60
atcataataa attaacccat acagatctga agcctgaaaa tattttgttt gtgaagtctg 120
actatgtagt caaatataat tctaaaatga aacgtgatga acgcacactg aaaaacacag 180
atatcaaagt tgttgacttt ggaagtgcaa cgtatgatga tgaacatcac agtactttgg 240
tgtctacccg gcactacaga gctcccgagg tcattttggc tttaggttgg tctcagcctt 300
gtgatgtttg gagcataggt tgcattctta ttgaatatta ccttggtttc acagtctttc 360
agactcatga tagtaaagag cacctggcaa tgatggaacg aatattagga cccataccac 420
aacacatgat tcagaaaaca agaaaacgca agtattttca ccataaccag ctagattggg 480
atgaacacag ttctgctggt agatatgtta ggagacgctg caaaccgttg aaggaattta 540
tgctttgtca tgatgaagaa catgagaaac tgtttgacct ggttcgaaga atgttagaat 600
atgatccaac tcaaagaatt accttggatg aagcattgca gcatcctttc tttgacttat 660
taaaaaagaa atgaaatggg aatcagtggt cttactatat acttctctag aagagattac 720
ttaagactgt gtcagtcaac taaacattct aatatttttg taaacattaa attatttgt 780
acagttaagt gtaaatattg tatgttttgt atcaatagca taattaactt gttaagcaag 840
tatggtcttg ataatgcatt agaaaaatta aaattaattt ttctttttga aattaccatt 900
tttaaatacc tttgaaatat cctttgtgtc cagtgataaa tgtgattgat cttgcctttt 960
gtacatggag gtcacctctg aagtgatttt ttttgagtaa aaggaaatct tgactacttt 1020
atattcttaa aggaatattc tttatatact tcaaatttag aacttaactt taaaagtttt 1080
tcttctgtaa ttgttgaacg ggtgattatt attaactcta gataagcagg tactagaaac 1140
caaaactcag aaaatgttta ctgttagaat tctattaaat tttaagtgtt gtattctttt 1200
tcattgggtg atgtcagggt gataaccaga cattcatgga aaggcatgca gtttgtccat 1260
tgtgacagtt tgtttaataa aaccacatac acccttaaa aaaaaaaaaa aaaaaaaaaa 1320
aaa 1323

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 00/00342

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[7]: C07K 14/435, 14/475, 14/705, A61KI 38/18, 38/17, A61P 3/04, 5/04, C12N 15/12, C12Q 1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN:(CA, MEDLINE, WPIDS, BIOSIS) KEYWORDS: LEAN, OBSES HYPOTHALAMUS, PROTEIN, ANTI?, LIGAND, BIND?"
ANGIS - BLASTN, FASTA (SEQUENCE SEARCH)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99/23217 A (International Diabetes Institute and Deakin University) 14 May 1999 See whole document.	1-17
X	Molecular Endocrinology (Endocrine Society, US) Vol.11.No 5, May 1997 Kesterson, RA et al., "Induction of neuropeptide..." pages 630-637 See abstract	1, 8, 14-16
X	Hormone And Metabolic Research Vol.28, No. 12, December 1996 Campfield, L.A, et al., "The OB protein..." pages 619-632, see abstract.	1, 8, 14-17

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
01 June 2000

Date of mailing of the international search report
21 JUN 2000

Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200
WODEN ACT 2606 AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No.: (02) 6285 3929

Authorized officer
J.G. HANSON
Telephone No.: (02) 6283 2262

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 00/00342

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	International Journal of Obesity Vol. 23, No 3, March 1999 Scacchi M et al , "Growth hormone in..." pages 260-271 See abstract and page 261, column 2, last para.	1, 8, 14-17
X	Peptides 1995 16(4) Frankish H.M et al. "Neuropeptide Y , the hypothalamus...", pages 757-771 See page 760, para. headed Neuropeptide Y: An Introduction and page 763	1, 8, 14-17
X	American Society For Microbiology Vol.11, No. 1, 1991, Howell, BW et al., "STY, a tyrosine-posphorlating enzyme...", pages 568-572 See Fig. 2 page 569	1-13
X	The Journal of Biological Chemistry Vol.270, No. 37, issued 15 September 1995 Duncan, PI, et al, "Alternative Splicing of..." Pages 21524-21531. See Fig 1 page 21526	1-13
X	The Biochemical Journal Vol. 326, pt. 3, 1997 Nayler, O., et al.. "Characterization and comparison of four...", pages 693-700 See abstract	1-13
X	Journal of Molecular Biology Vol. 244, 1994 Hanes, JJ, et al. "Characterization by cDNA cloning...", pages 665-672. See Figures 1 and 2 pages 667 and 668.	1-13

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 00/00342

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	99/23217	AU	10112/99

END OF ANNEX